

FIGURE 1: (A) Restriction map of pALA–D. R = Rsal, P = Pstl. Fragments A – D are labeled above the line, with the nucleotide lengths indicated beneath. There is a single <u>Smal</u> site in fragment D. (B) Branch migration of displacer (open rectangle), bound to linker (filled rectangle), into a recipient duplex with a four base 3'–overhang (<u>Pstl</u> end of fragment B). Shown below is the conversion between the displacer–linker duplex bound to the 3' overhang only (left) and following complete branch migration (right). (C) Maximum displacement with specific pALA–D fragments. m = the maximum number of base pairs which can be formed between the displacer and the complementary recipient strand.

٠,

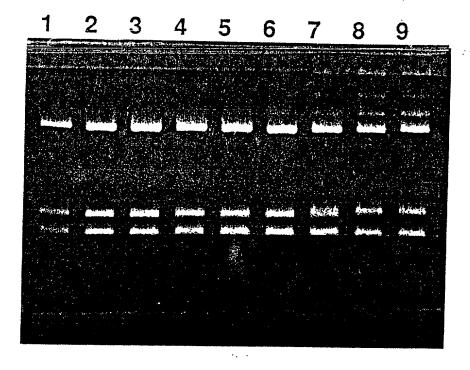


FIGURE 2: Capture reaction of P–D–BrdC plus P–L–dC. UV fluorogram of 1% agarose gel. Lane 1: Rsal/Pstl digested pALA–D (200ng). A, B, C, and D refer to fragments shown in Fig. 1. Lanes 2–9: products following ligation in the presence of P–D–BrdC (6 μ g/ml), P–L–dC (2 μ g/ml), and 5 U/ml ligase for 1, 2, 4, 8, 16, 32, 64, and 128 min, respectively.

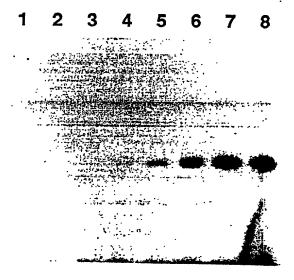


FIGURE 3: Autoradiogram of Fig. 2. Lanes 1–8 correspond to the radiolabeled lanes 2–9 of Fig. 2.

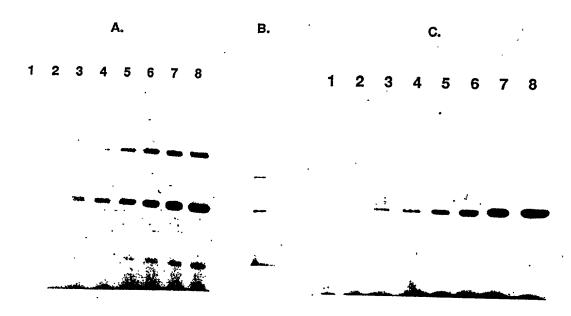


FIGURE 4: (A) Autoradiogram similar to Fig. 3, but with higher ligase concentration and P-D-dC replacing P-D-BrdC. (B) An early time point in an autoadiogram identical to Fig. 3 except using P-D-BrdC-E(10) replacing P-D-BrdC. (C) Autoradiogram identical to Fig. 3 except using P-D-BrdC-E(24) replacing P-D-BrdC.



FIGURE 5: Cloning. Autoradiogram of sequencing gel showing the region of incorporated displacer (bold) and linker (underlined) sequences.

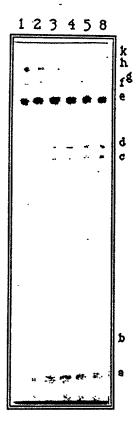


FIGURE 6: Partial Digest Mapping. BCR using pALAD-G4, a derivative of pMS19 containing a genomic fragment of human ALAD, and displacer-linker duplex S-D-BrdC and S-L-dC was followed by partial digestion with Sau3A1. Lanes 1, 2, 3, 4, 5 and 8: partial digestion products formed at 1, 2, 3, 4, 5 and 8 minutes, respectively. Bands a-k are partial digest bands of the sizes expected: 300, 406, 1538, 1598, 2706, 2731, 2748, 3198, and multiple large bands produced by sites within the vector, respectively.

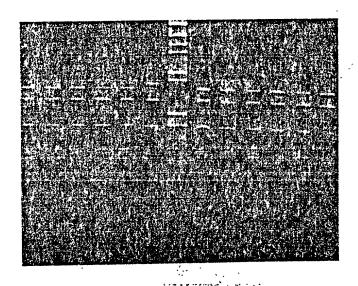


FIGURE 7: Triplex enhanced branch-migration mediated linker capture. Lanes 1–6: pMS19, cut with Ncil and Sall, incubated with BT-D-McdC-1, BT-L-dC-1 and T4 DNA ligase as described in the text, for 0, 1, 3, 10, 30 and 120 min. Lane 7: Molecular weight markers of lambda DNA cut with Avall. Lanes 8–13: pMS19, cut with Ncil and Sall, incubated with B0-D-McdC-1, BT-L-dC-1 and T4 DNA ligase as described in the text, for 0, 1, 3, 10, 30 and 120 min.